GUIDE

TO

THE PRACTICAL STUDY OF HISTOLOGY

FOR MEDICAL STUDENTS

BY

D. NOËL PATON, M.D.

PROFESSOR OF PHYSIOLOGY, UNIVERSITY OF GLASGOW

AND

G. HERBERT CLARK, M.B., D.P.H.

LECTURER IN PHYSIOLOGY, UNIVERSITY OF GLASGOW

SECOND EDITION

GLASGOW

JAMES MACLEHOSE AND SONS

PUBLISHERS TO THE UNIVERSITY

1914

PRICE ONE SHILLING NETT

GUIDE

TO

THE PRACTICAL STUDY OF HISTOLOGY

FOR MEDICAL STUDENTS

BY

D. NOËL PATON, M.D.

PROFESSOR OF PHYSIOLOGY, UNIVERSITY OF GLASGOW

AND

G. HERBERT CLARK, M.B., D.P.H.

LECTURER IN PHYSIOLOGY, UNIVERSITY OF GLASGOW

SECOND EDITION

GLASGOW

JAMES MACLEHOSE AND SONS

PUBLISHERS TO THE UNIVERSITY

1914

GUIDE TO THE PRACTICAL STUDY OF HISTOLOGY.

INTRODUCTION.

The practical Study of Histology may be divided into three parts :

1st—The methods of preparing specimens for microscopic examination (pp. 1 to 19).

2nd—The method of examining and reporting on specimens (pp. 20 and 21).

3rd—The structure of the normal tissues, organs and fluids of the body. This must be learned from the specimens prepared and examined (pp. 22 to 31).

PART I.

METHODS OF PREPARING SPECIMENS.

A. FLUIDS.

1. **Opaque Fluids** (such as blood and milk).—These usually contain solid particles uniformly distributed.

(*a*) *Examination in fresh condition.* A drop of the fluid is placed on a microscopic slide, covered with a clean cover-glass, and examined first, unstained ; second, after staining or treatment with reagents if necessary. (See p. 10.)

(*b*) *Permanent preparations* may often be made by spreading a thin layer on a cover-glass, drying, fixing staining, and mounting in Canada balsam. (See Blood Films, p. 13.)

2. **Transparent Fluids.**—These have usually few solid particles, and they may (*a*) be set aside to allow of the precipitation of solid matter as a deposit, or (*b*) be centrifugalised to accelerate precipitation.

Some of the deposit is taken in a pipette, placed on a slide, covered and examined.

Some deposits may be *preserved* either by drying, staining, and mounting in balsam, or, in the case of some urinary crystals, by the addition of glycerine jelly.

B. SOLIDS.

Solid structures should be examined, (*A*) in the fresh condition ; (*B*) after preparation.

A. To examine in the **fresh condition** the following methods may be employed :

1. *Scraping.*—If the tissue is soft, the surface may be *scraped*, the scrapings placed on a clean cover-glass, and another cover-glass pressed upon it. The two cover-glasses are then slid along one another till they era separated, and the thin layer of tissue left upon one is mounted in a drop of some indifferent solution, such as a 0·75 per cent. solution of common salt.

If necessary, such preparations may be stained with aqueous solutions of aniline dyes, such as methylene blue. Sometimes they may be preserved by drying, fixing, staining, and mounting in balsam, in the same manner as blood films.

2. *Teasing.*—If the tissue is fibrous, a small piece may be *teased* out with needles, and examined in a drop of salt solution, either unstained or stained.

3. *Section Cutting.*—By *freezing* the tissue, thin slices may be cut with a sharp razor (p. 7), floated in a 5 per

cent. formalin solution, spread upon a microscope slide, and examined unstained or stained. Such preparations may afterwards be preserved.

Such methods of examination often give much information as to the nature of the structures examined, and are sometimes sufficient. In every case they afford indications of the best mode of preparing and carrying out the further examination.

B. In order to make **permanent preparations**, it is in most cases necessary to carry out the following processes in order: (1) Fixing, (2) Dehydrating and hardening, (3) embedding in some material which facilitates section-cutting, then (4) cutting, (5) staining, (6) mounting in some preservative medium.

GENERAL METHODS.

1. FIXING.

Tissues must be taken as soon as possible after the animal is killed, to avoid post-mortem changes, cut into small pieces not more than a quarter of an inch each way, to allow the fluid to penetrate rapidly, and thrown into a relatively large quantity of the fixing fluid.

1. *Formalin* (a 40 per cent. watery solution of formaldehyde). This is generally used in solutions of from 5 to 10 per cent. in 0·75 per cent. sodium chloride solution, or in alcohol or in a saturated solution of picric acid. It acts very rapidly, penetrates large pieces of tissue, and does not overharden.

Tissues after lying in formalin solution till they are penetrated—a few minutes—may be frozen, and at once cut and stained.

2. One of the most generally useful fixing fluids is a *saturated solution of perchloride of mercury in water or in* 0·75 *per cent. salt solution*. The solution must be saturated

while boiling, and then allowed to cool. Tissues should remain in it until they are thoroughly penetrated, which will usually be in something under twenty-four hours.

3. *Bichromate of potash* (2 to 5 per cent. in water) is a very weak fixative, but an excellent hardening reagent, especially for the central nervous system. It is generally used with sulphate of soda, as Müller's fluid. (Appendix.)

In most cases it is necessary to *wash out the excess of the fixing agent*, as the presence of many of these substances in the tissue interferes with staining. Twenty-four hours in running water is usually sufficient for this purpose, and that time should rarely be exceeded, as tissues are apt to become soft if kept longer in water. With weak fixatives, like picric acid, it is better, indeed, to transfer the object directly to alcohol.

2. DEHYDRATING.

In most cases the process of dehydrating is carried out by means of alcohol. This dehydration is a necessary step in the embedding processes most commonly used—paraffin and celloidin. The replacing of water by alcohol should take place gradually, in order that the tissues may shrink as little as possible. Therefore the object is passed through alcohols of gradually increasing strength, first for a few hours in 30 per cent. alcohol, then for some hours in 50 per cent. alcohol, next for the same time in methylated spirit, and last in absolute alcohol.

Where corrosive sublimate has been used as the fixing agent, the traces of it which have not been removed by washing the tissues may be cleared away by adding a little tincture of *iodine* to the alcohols used for hardening.

3. EMBEDDING AND SECTION-CUTTING.

Few objects are sufficiently homogeneous in texture to allow of good sections being cut without further preparation, and it is necessary, therefore, to infiltrate

the tissue with some substance which will allow it to be treated as if it were a solid block, by filling the minutest cavities, and which will accurately preserve the relations of parts. This may be done, either (*a*) by means of a substance which is fluid at ordinary temperatures and becomes solid by freezing, such as a thick solution of dextrin ; or (*b*) by a substance which is fluid when heated and becomes solid on cooling, such as paraffin ; or (*c*) by a substance which can be dissolved by solvents whose evaporation leaves it solid, such as celloidin or photoxylin.

1. *The Dextrin Method.*—This is mainly employed for tissues which are too brittle to be cut in paraffin, or when rapid examination is required. Every trace of alcohol must be washed out of the specimen by twenty-four hours' soaking in running water, as alcohol interferes with the freezing. It is then soaked in a thick solution of dextrin for twelve hours or more, according to the size of the piece taken, placed on the pedestal of a freezing microtome, the dextrin painted round it, and the whole is frozen by the evaporation of ether or by compressed carbon dioxide. The mass should cut like cheese. The sections are soaked in water to remove the dextrin, and may either at once be stained, mounted and examined, or preserved in spirit for future use.

2. *The Paraffin Method.*—(1) The tissue, thoroughly dehydrated in absolute alcohol, is *cleared* in a solvent of paraffin, such as cedar oil, chloroform, or xylol, which removes the alcohol and renders it possible for the paraffin to penetrate the object. (2) It is then placed in melted paraffin, in an oven with a water-jacket, which is kept at a constant temperature, just above the melting-point of the paraffin used. If this be 50° C., the oven should be regulated to 52° C. *Tissues are spoilt if they are heated above* 60° C. The object is kept in this until the clearing agent used has diffused out into the paraffin, and has either evaporated or been removed by several changes of paraffin. Twenty-four hours is usually a sufficient time for all but the densest tissues. (3) The object is then placed in a

suitable dish and arranged in the paraffin. The dish is then placed in cold water, so that the paraffin may cool rapidly and set homogeneously. (4) The block when cool is removed from the dish, trimmed to a rectangular shape, and (5) sections are cut with a microtome. For this purpose a good razor with a smooth edge is essential.

A complete series of sections may be cut. These sections are all of equal thickness, and the relation of different parts is perfectly preserved in them.

3. *The Celloidin Method.*—(1) The tissue is dehydrated in absolute alcohol ; (2) soaked in a mixture of absolute alcohol and ether ; (3) then transferred to a thin solution of celloidin or photoxylin in absolute alcohol and ether ; and (4) to two or three thicker solutions of celloidin or photoxylin, or the initial solution may be allowed to concentrate itself by slow evaporation, lasting over days or weeks. As soon as the mass of celloidin surrounding the object will no longer pour readily, the object is placed in a cell made by surrounding a block of wood with a strip of paper overlapping at one end, the cell thus formed filled with thick celloidin, and the alcohol and ether allowed to evaporate slowly in an atmosphere of chloroform vapour. When the surface of the celloidin or photoxylin has become firm, the whole is hardened in 85 per cent. alcohol. The block is then fixed to a microtome and cut by an obliquely-set knife sliding in a groove and kept wet with alcohol.

This method is employed for the central nervous system, for very large objects which are difficult to infiltrate with paraffin, and for objects, such as bone and skin, which are so brittle as to make it difficult to cut them in paraffin.

Objects embedded in celloidin may be re-embedded in paraffin and cut dry.

4. FIXING ON SLIDE.

The sections must next be *fixed to the slide* or to the cover-glass. This is best done by (1) floating the sections on warm water, at a temperature a good deal below the melting-point of the paraffin, when they flatten out, and can easily be floated on the slide. (2) The water between section and slide is driven off by very gentle heat, and the section adheres firmly to the slide. (3) The paraffin is removed by xylol, turpentine, or naphtha, and the section is passed through spirit into water, and then stained. Sections fixed in formalin do not adhere readily, and the slide may be first coated with a 10 per cent. solution of egg white in water.

CLASS ROUTINE.

For Freezing or Paraffin Process.

1. *Fixing.*
   24 hours in 10 per cent. solution of formalin in picric acid.
2. *Dehydrating and Hardening.*
   24 hours in each of (*a*) 30 per cent. alcohol.
   (*b*) 50 per cent. alcohol.
   (*c*) 75 per cent. alcohol.

A. For Freezing Process.

3. *Washing out Alcohol.*
   24 hours in tap water.
4. *Impregnation.*
   24 hours in dextrin.
5. *Embedding and Cutting.*

B. For Paraffin Process.

3. *Further Dehydration.*
   (*a*) Absolute alcohol - - - 24 hours.
   (*b*) ,, ,, - - - 24 hours.

4. *Clearing.*
   (*a*) Cedar wood oil - - - 24 hours.
   (*b*) ,, ,, - - - 24 hours.
5. *Impregnation.*
   (*a*) Melted paraffin in oven - - 6 hours.
   (*b*) ,, ,, ,, - - 2 hours.
6. *Embedding and Cutting.*

Rapid Paraffin Method.

Very small pieces of tissue are used.

1. *Fixing.*
   30 minutes in a mixture of two-thirds absolute alcohol and one-third glacial acetic acid.
2. *Dehydrating and Hardening.*
   30 minutes in absolute alcohol.
3. *Clearing.*
   15 minutes in xylol.
4. *Impregnation.*
   (*a*) Melted paraffin in oven - 15 minutes.
   (*b*) ,, ,, ,, - 15 minutes.
   (*c*) ,, ,, ,, - 15 minutes.
5. *Embedding and Cutting.*

5. STAINING.

Stains are used in order to differentiate the parts of a tissue, generally by staining the nuclei of one colour, and the cell-bodies and formed material in the section of another colour. That this can be done depends on the fact that nuclear chromatin has an affinity for basic stains, while cell protoplasm and formed material generally take up acid stains.

The difference between these two sets of stains is best seen in the aniline dyes, where **basic stains** are those salts formed of a colourless acid and a coloured base, while **acid stains** are salts formed of a coloured acid and a colourless base. Some of the more important basic aniline

stains are : Methylene blue, methylgreen, toluidin blue, fuchsin, gentian-violet. Carmine and hæmatoxylin, or hæmatein, act in many respects as basic stains. Some **acid stains** are eosin, orange, acid fuchsin and picric acid.

The stains most in use for general histological work are the aniline stains and the combination of hæmalum with eosin.

1. *Hœmalum and Eosin.*—Hæmatein is usually dissolved in alcohol or glycerine, and made up with a watery solution of alum—hæmalum. (Appendix.)

Staining takes place rapidly, and as diffuse-staining is apt to occur, it is usual to wash the section for some time in water. The section may then be stained in a watery solution of eosin. *Van Gieson's stain* (a mixture containing picric acid and acid fuchsin) may be used as a counter stain in place of eosin.

2. *Aniline Stains.*—These may be used in different ways.

(*a*) Overstain the tissue with a strong solution of a basic stain in alcohol, or water, or aniline water, and then wash out the excess with alcohol plain or acidulated or with a solution of one of the acid stains, which substitutes itself for the basic stain in everything but the nuclei. The process of substitution must be stopped at exactly the point required, and this is usually done by rapid dehydration and mounting in balsam.

Example—Nissl's Method.—This is used to bring out the Nissl's granules in neuron cells. The tissue, fixed in perchloride of mercury, is stained for from half to one hour in a saturated solution of toluidin blue. It is then partially decolorised, dehydrated in alcohol, cleared and mounted in balsam.

(*b*) Employ a combination of several stains in a single solution. The section is stained in this, and the different dyes are taken up by the structures which have a special affinity for them. The best known of these mixtures is the *Triacid* or *Ehrlich-Biondi* stain, which contains methyl-

green as the basic stain, and the two acid stains—acid fuchsin and orange.*

3. *Picrocarmine* or picrocarminate of ammonia, a salt formed by the decomposition of an ammoniacal solution of carmine and a solution of picric acid, is a double stain. Nuclei become red, formed material either pink or yellow —*e.g.* white fibrous tissue becomes pink, elastic fibres yellow. It is used in watery solution, and should be allowed to act on the section as long as possible, or its action may be hastened by warmth. Sections stained with it are usually mounted in a glycerine medium, such as Farrant's medium.

6. MOUNTING IN A PRESERVATIVE MEDIUM.

The object of mounting is to enclose the preparation with some material which will render it of a proper degree of transparency, which will preserve the stains which have been used, and which, though fluid when used, will set afterwards so as to ensure that the section and cover-glass will not be displaced.

1. *Glycerine* is a useful temporary mount, but does not set. Farrant's medium partly removes this difficulty. Its advantages are that sections can be brought into it directly from water or a watery stain, and that after some time it sets fairly well. But most stains rapidly fade in it, and in practice only sections stained with picrocarmine are usually mounted in it.

2. *Canada balsam* dissolved in xylol is the medium most in use. It renders tissues more transparent than the glycerine media, it preserves tissues and stains, and sets rapidly and firmly. Sections to be brought into it must—(1) be dehydrated with alcohol, (2) cleared with xylol, clove oil, cedar oil, or some other of the clearing agents, and (3) then brought into the balsam and covered.

*This is best purchased in powder from one of Grübler's agents.

SPECIAL METHODS.

1. EXAMINATION OF BLOOD.

A. Blood Films.

Clean the finger behind the nail, or the lobe of the ear with alcohol. Pass a clean needle through the flame of a burner, and sharply prick the finger or ear.

Spread the drop of blood in a thin layer between two **clean** slides or cover-glasses by drawing them gently apart. Dry them in air. Place them face downwards in methyl alcohol for four minutes and wash them in water.

(*a*) *Eosin and Methylene Blue.*—To stain the film immerse in a saturated watery solution of eosin for five minutes. Wash in water. Dip in a saturated solution of methylene blue for half a minute. Wash in water. Dry thoroughly by warming and mount in balsam.

(*b*) *Jenner's Stain* (see Appendix).

Previous treatment with methyl alcohol is unnecessary with this stain.

Immerse the slide or cover-glass with dried film in the stain for 2 minutes. Pour off excess. Next add water drop by drop until a metallic film appears on the surface of the stain. Stain for 5 minutes. Rinse in *distilled* water till the film has a pink colour.

Dry and mount as above.

B. Weigert's Method for Staining Fibrin.

(1) Harden in alcohol.
(2) Stain for 5 to 15 minutes in a strong aniline-water solution of gentian-violet.
(3) Wash in 0·75 per cent. salt solution.
(4) Dry on a slide.

(5) Place for two or three minutes on a slide in a solution of iodine 1 part, potassium iodide 2 parts, water 100 parts.
(6) Dry with blotting paper.
(7) Decolorise in aniline oil 2 parts, xylol 1 part.
(8) Remove the aniline-xylol with xylol.
(9) Mount in Canada balsam.

C. Enumeration of Cells.

Thoma-Zeiss Hœmocytometer.

Erythrocytes.

1. *Dilution of Blood to Definite Proportion.*—Puncture the finger or lobe of the ear with a sharp needle. Suck the blood thus obtained into the pipette up to the mark 0·5 on the stem. Rapidly, before the blood has time to clot, dry the point, and suck Hayem's solution (Appendix) into the pipette up to the mark 101, mixing thoroughly by gently shaking the pipette. The dilution is now 1 to 200.

2. *Enumeration of Corpuscles in a Definite Volume of Diluted Blood.*—Blow a few drops of the mixture out of the capillary part of the pipette and then place a small drop on the platform in the centre of the counting slide. Cover with a cover-glass. *In putting on the cover-glass, care must be taken to allow none of the mixture to pass into the trench which surrounds the platform.* No air bubbles must be present.

Allow a minute to elapse before commencing to count.

Count three sets of sixteen squares.

Each ruled square has resting upon it, when the cover glass is in position, $\frac{1}{4000}$ c.mm. of the diluted blood. Hence the number of corpuscles in 1 c.mm. of diluted blood can be taken as the average number of corpuscles on one square multiplied by 4000.

The dilution of the blood was 1 to 200 so that by further multiplying by 200 the number of corpuscles per c.mm. of *undiluted* blood may be found.

Leucocytes.

Collect the blood to the mark ·5 in the larger pipette marked 11 at the top of the bulb and suck a 0·3 per cent. glacial acetic acid solution, tinged with methyl violet to stain the leucocytes, to the mark 11, so that the dilution is 1 to 20.

Count the white corpuscles in all the 256 small squares. The average per square multiplied by 4000, as above, gives the number of leucocytes per c.mm. of diluted blood. As the dilution is 1 to 20, further multiplication by 20 will give the total number per c.mm. of *undiluted* blood.

2. DISSOCIATING TISSUES.

It is sometimes necessary to make a tissue easily disintegrated, so that the individual elements may be separated from one another. This may be done by macerating it in a solution of one-third alcohol (Ranvier's alcohol) or in a 2 per cent. solution of yellow chromate of potash.

3. STAINING WITH NITRATE OF SILVER.

This is generally used in order to demonstrate the outlines of epithelial or endothelial cells or connective tissue spaces, and its use can be best described as it is carried out with the omentum. This is taken *fresh*, and is placed in a 1 per cent. solution of nitrate of silver in the dark until the membrane turns grey. It is then washed in distilled water and exposed to sunlight in 50 per cent. alcohol until brown.

4. CAJAL'S METHOD FOR NEURON PROCESSES.

By this method the processes are rendered opaque by the deposition of silver.

(1) Small pieces of tissue, not more than 3 mm. cube, are placed in alcohol with 1 per cent. of ammonia and kept for 24 hours at a temperature of from 30° to 35° C.

(2) After washing in distilled water they are removed to a 1 to 2 per cent. solution of silver nitrate and again kept at the same temperature for 5 to 8 days.

(3) After thoroughly washing in distilled water the tissue is transferred to a reducing solution of pyrogallic acid (Appendix) for 24 hours. A yellowish brown colour on the cut surface shows that the process has been properly carried out.

(4) After washing in distilled water the tissue is dehydrated and cut in paraffin or celloidin.

The process must be carried out in the dark until after the reduction in the pyrogallic acid solution is completed.

5. GOLGI'S METHOD FOR NEURONS.

Cox's Modification.

With this method, not the substance between the cells, but the cells themselves are impregnated with chromate of mercury. It is used mainly for the nervous system. Small pieces of tissue are taken fresh, and are left for two or three months in a mixture of chrome salts and a mercuric salt. (Appendix.)

Thick sections are cut with the free hand and put into a saturated solution of lithium carbonate for 1 hour, washed in water, dehydrated, cleared rapidly, and mounted in balsam without a cover-glass.

The neuron cells and their processes are impregnated with the chromate of mercury, and appear black by transmitted light.

6. WEIGERT'S METHOD FOR NERVE FIBRES.

Wolter Kulschitsky's Modification.

This method is used for staining the white sheath of nerve fibres.

The tissue is fixed in Müller's fluid, hardened in alcohol, and embedded in celloidin.

Sections are stained for 24 hours at a temperature of 40° C. with Kulschitsky's hæmatoxylin. (Appendix.)

They are then immersed in Müller's fluid until black, transferred to a 0·25 per cent. solution of potassium permanganate for 30 seconds. The sections become brown. They are then differentiated in Pal's fluid (Appendix), washed in water, dehydrated, cleared and mounted in Canada balsam.

7. MARCHI'S METHOD FOR NERVE FIBRES.

This method is used for tracing the course of degenerated white nerve fibres, and it depends on the fact that the myelin substance while disintegrating, stains black when treated with a mixture of a chrome salt and osmic acid. The tissue is kept in Müller's solution for 8 days and then placed for 4 days in a mixture composed of 2 parts Müller's fluid and 1 part of a 1 per cent. solution of osmic acid. It is then carried through alcohols of increasing strengths and cut in celloidin or paraffin.

Busch secures better penetration by using osmic acid 1 part, sodium iodate 3 parts, in 100 of water, instead of osmic acid and Müller's solution.

8. METHYLENE BLUE METHOD FOR NEURON FIBRES.

Spread out the tissue as thin as possible and wash in normal salt solution at 37·5° C. Place it in an open dish in an incubator at 37·5° C. and immerse in 1 in 10,000 saline solution of Grübler's Bx methylene blue.

Keep it moist by adding a few drops from time to time.

Examine with a microscope every 15 to 30 minutes.

The stain takes from 2 to 6 hours and stains the nervous elements in the following order :

(1) Fine terminations.
(2) Nerve fibres.
(3) Nerve cells.

Specimens may be fixed by placing them in a saturated solution of ammonium picrate for an hour.

Mount in glycerine to which a drop of ammonium picrate has been added.

9. GOLD CHLORIDE METHOD FOR NEURON FIBRES.

Pieces of fresh tissue or frozen sections are placed in :

(1) 25 per cent. formic acid for two minutes.

(2) 5 per cent. gold chloride solution for 20 to 30 minutes.

(3) 25 per cent. formic acid solution for 24 hours in the dark.

The specimens are mounted in glycerine acidulated with 1 per cent. formic acid.

10. STAINING FOR IRON.

The detection of the products of the disintegration of hæmoglobin may be effected by taking advantage of the fact that iron in simple compounds gives a colour reaction with certain reagents.

(1) A scraping of the tissue—*e.g.* spleen—spread upon a slide, is treated with *ammonium sulphide*, covered and examined at once. The iron is dark green.

(2) Sections are washed, then placed for 10 minutes in a mixture of 4 per cent. *potassium ferrocyanide* and 2 per cent. *hydrochloric acid*. The iron is stained blue. The sections may then be washed, counter-stained for 10 minutes with carm-alum, dehydrated, cleared and mounted.

11. STAINING FOR GLYCOGEN.

Glycogen stains a dark brown with *iodine*.

(1) Scrapings of fresh liver may be treated with a solution of iodine in iodide of potassium and examined at once.

(2) Sections of tissues hardened in alcohol may be

stained with iodine as above, dehydrated, cleared with alcohol and xylol containing iodine, and mounted in balsam.

12. STAINING FOR FAT.

Sections of tissues frozen directly or after fixing in formalin are generally used :

(1) *Osmic acid.* The sections are placed in 0·5 per cent. solution of osmic acid for about five minutes, then washed.

(2) *Sudan III.* The sections are placed in a saturated solution of sudan in 80 per cent. alcohol for 10 minutes, then decolorised in 80 per cent. alcohol—usually for about 3 minutes, and mounted in Farrant's medium. They may be counter-stained with hæmalum.

13. STAINING ELASTIC FIBRES.

Sections are stained for 20 minutes to one hour (warmed slightly) in resorcin-fuchsin (Appendix), washed in alcohol for 5 to 10 minutes, cleared with xylol, and mounted in Canada balsam. Elastic fibres are stained dark blue.

PART II.

METHOD OF EXAMINING SPECIMENS.

(A) Examine fresh and unstained (p. 3 *et seq.*). (B) Examine prepared and stained (p. 5 *et seq.*).

1. First with the **naked eye**, and then with the low power, study the general arrangement, and distinguish the separate parts to be studied, making a sketch or plan and an inventory of the various parts to be examined in detail.

2. Study the separate parts in detail with the **low power :**

(i) *Shape.* (*a*) Length, (*b*) breadth, (*c*) depth (thickness). (ii) *Colour.* (iii) *Outline.* Sharply or not sharply defined—double or single contour. (iv) *Texture.* Homogeneous, granular, fibrous, etc. (v) *Size.* (*a*) Compare with Human Erythrocyte, which is 7·7 micros. in diameter, under the same power ; (*b*) measure with the micrometer.

Measurement of Objects.

A. *With Micrometer Scale.*

Prepare a micrometer scale by laying a sheet of white paper close to the right side of the base of the microscope and placing a stage micrometer ruled in divisions of $\frac{1}{10}$ and $\frac{1}{100}$ mm. under the objective. With the left eye look down the tube of the microscope, keeping the right eye open, and the lines on the micrometer will appear to be projected on to the paper. Mark these lines with a pencil.

The spaces correspond to $\frac{1}{10}$s and $\frac{1}{100}$s of a mm. When an object has to be measured place it under the objective and treat it in the same way as the stage micrometer, and its image will appear projected on the spaces marked on the paper, and it may thus be measured.

B. *With the Eye-piece Micrometer.*

Adjustment of Eye-piece Micrometer.—(*a*) Place the disc of glass on the ledge in the middle of the eye-piece so that the ruling is seen when the eye-piece is in position.

(*b*) Place a stage micrometer on the stage of the microscope and bring the ruling into focus.

(*c*) Now, by pulling out or pushing in the draw tube, bring one, two or more of the spaces of the eye-piece micrometer to correspond to a space of the stage micrometer. If the spaces on the stage micrometer are $\frac{1}{10}$ mm., then the length to which a space on the eye-piece micrometer corresponds may be calculated.

Points which cannot be made out with the low power are then to be examined with the **high power.**

Drawings of the whole specimen or of its various parts must be made under the low power, and if necessary of various parts under the high power.

PART III.

THE STRUCTURE OF THE NORMAL TISSUES AND ORGANS.

The structure of the tissues, organs and fluids is to be studied practically on a series of specimens prepared by the student or given out during the session. Drawings and descriptions of each of these must be made.

In *revising* this part of the work, any of the standard text-books of Histology may be used.

LIST OF SPECIMENS USED IN STUDYING TISSUES AND ORGANS.

When not otherwise stated, the specimens are stained with Hæmalum and Eosin.

Those in italics are unstained and are not permanent preparations.

A. CELL.

1. T.S. Pregnant Uterus (Rabbit).

B. TISSUES.

Epithelium.

2. *Epithelial Scales in Saliva* (*Human*).
3. T.S. Œsophagus (Dog).
4. Epithelium from Bladder, dissociated (Human).
5. T.S. Urinary Bladder (Dog).

6. S. Lung, silvered (Kitten).
7. Ciliated Epithelium, dissociated (Ox). Picrocarmine.
8. T.S. Trachea (Rabbit).
9. *Gill of Mussel.*
10. T.S. Small Intestine (Cat).
11. Columnar Epithelium, dissociated (Ox). Picrocarmine.
12. *Salivary Gland, teased (Rabbit).*
13. S. Salivary Gland (Cat).
14. S. Pancreas (Dog).
15. L.S. Kidney (Rabbit).

Connective Tissues.

A. Fibrous Tissue.

16. T.S. Snout of Fœtal Pig.
17. T.S. Tendon (Sheep). van Gieson.
18. Tendon, teased (Rat's tail). Hæmalum.
19. Lig. Nuchæ of Ox, teased. Unstained.
20. T.S. Lig. Nuchæ of Ox. van Gieson.
21. *Subcutaneous Tissue, teased.*
22. Subcutaneous Tissue for Fat Cells (Rabbit). Sudan III.
23. L.S. Corneo-sclerotic Junction (Rabbit).
24. Endothelium of Omentum, silvered (Kitten).
25. S. Lymph Gland of Fasting Animal (Cat).

B. Cartilage.

T.S Snout of Fœtal Pig (16).

26. L.S. Foot of Fœtal Pig.
27. Costal Cartilage (Human). Hæmalum and van Gieson.
    T.S. Trachea (8).
28. T.S. Intervertebral Disc (Ox). Hæmalum.
29. L.S. Epiglottis. Hæmalum and van Gieson.

C. Bone.

T.S. Snout of Fœtal Pig (16).
L.S. Foot of Fœtal Pig (26).

30. T.S. Compact Bone, softened (Dog). Hæmalum and van Gieson.

31. L.S. Compact Bone, softened (Dog). Hæmalum and van Gieson.

Demonstrations of Unsoftened Bone.

Muscle.

32. Visceral Muscle, teased (Ox). Picrocarmine
T.S. Small Intestine (10).
33. *Skeletal Muscle, fresh teased (Frog).*
34. Skeletal Muscle, teased (Frog). Eosin.

From Alcohol.

35. Skeletal Muscle, teased (Crab). Eosin.
36. T.S. Tongue (Rabbit)
37. T.S. Tail of Salamander.
38. Heart Muscle, teased (Human). Hæmalum.
39. L.S. Heart (Rat).

Nerve.

40. *White Nerve Fibres, fresh, teased (Rabbit).*
41. White Nerve Fibres, osmic acid, teased (Rabbit).
42. T.S. Vago-Sympathetic Nerve, osmic acid (Dog).
43. T.S. Nerve (Rabbit).
44. T.S. Spinal Ganglion, stained Nissl's method (Cat).
45. T.S. Spinal Cord (Cat).
46. Cortex Cerebri, stained Golgi's method (Cat).

Blood.

47. *Blood, Human, fresh.*
48. Blood Film, Jenner's Stain, Human.
49. *Blood of Frog, fresh.*
50. Blood Film, Jenner's Stain, Frog.
51. Spleen, fresh, film. Eosin and Methylene Blue.
52. S. Spleen (Cat).
Lymph Gland (25).
53. T.S. Bone Marrow (Rabbit). Eosin and methylene blue.
54. Bone Marrow. Oxidase reaction.

C. ORGANS.

Endocrinus Glands.

55. S. Thymus (Guinea Pig).
56. S. Thyroid and Parathyroid (Dog).
57. S. Suprarenal. Chrome salt.
58. S. Suprarenal (Dog).
59. Pituitary (Sheep).
    Pancreas (14).
60. Testis Epididymis (Rat).
61. Ovary (Rabbit).

Circulation.

    L.S. Heart (39).
    Heart Muscle, teased (38).
62. T.S. Aorta.
    Small Arteries and Veins (52, etc.).
63. Capillaries of Pia Mater (Sheep). Hæmalum.
64. T.S. Mesentery, showing vessels (Rabbit).
65. Small Intestine, injected, surface view (Rabbit).

Respiration.

    T.S. Pig's Snout (16).
    T.S. Trachea (8).
    Ciliated Epithelium (7).
    Epiglottis (29).
66. S. Lung (Cat).
67. S. Lung (Rabbit), Resorcin Fuchsin.
68. S. Lung, injected (Rabbit)
    Lung, silvered (6).
69. S. Lung, fœtal (Human).

Digestion.

    T.S. Tongue (36).
70. L.S. Tooth, softened (Dog). van Gieson.
    Demonstrations of Unsoftened Tooth.
    T.S. Pig's Snout (16).

71. Tonsil (Dog).
    T.S. Œsophagus (3).
    Salivary Gland (13).
72. L.S. Stomach, cardiac end (Dog).
73. L.S. Pyloro-duodenal Junction (Cat).
    Pancreas (14).
74. Liver, fresh, teased. Stained iodine.
75. T.S. Liver of Pig.
76. T.S. Liver of Rabbit, injected.
    T.S. Small Intestine (10).
    Small Intestine, injected, surface view (64).
77. T.S. Small Intestine, Peyer's Patch.
78. T.S. Large Intestine (Dog).
79. T.S. Vermiform Appendix (Rabbit).

Nervous System.

White Nerve Fibres, osmic acid (41).
T.S. Nerve (43).
T.S. Spinal Ganglion (44).
T.S. Spinal Cord (45).

80. T.S. Spinal Cord, stained Weigert's method (full time fœtus).
81. T.S. Spinal Cord, stained Marchi's method (degeneration) (Human).
82. T.S. Cerebrum, Nissl's method (Mouse).
    T.S. Cortex Cerebri, Golgi's method (46).
83. T.S. Cerebellum (Cat).
84. T.S. Cerebellum, Cajal's method (Rabbit)
    Corneo-sclerotic Junction (Rabbit) (23).
85. T.S. Back of Eye, with Retina (Rabbit).
86. L.S. Eyelid (Human).
    Pacinian Bodies (90).

Excretion.

87. *Kidney, fresh, teased.*
    Kidney (15).
88. T.S. Kidney, injected (Dog).

89. T.S. Ureter (Cat).
    T.S. Urinary Bladder (5).
    Bladder Epithelium, dissociated (4)
90. S. Skin from tip of finger (Human).
91. *Hair, in Caustic Potash* (*Human*).
92. T.S. Scalp (Human).
93. L.S. Developing Nail (Human).

Reproduction.

    T.S. Testes and Epididymis (60).
94. T.S. Prostate (Dog).
    T.S. Ovary (61).
95. T.S. Fallopian Tube (Human).
96. T.S. Uterus (Human).
    T.S. Placenta (Human).
97. Pregnant Uterus of Rabbit (1).
98. T.S. Umbilical Cord (Human).
99. Mammary Gland (Rabbit).

APPENDIX.

Flemming's Solution.

1 per cent. chromic acid,	15 parts.
2 ,, osmic acid,	4 ,,
Glacial acetic acid,	1 part.

Phloroglucin-Nitric Acid Solution for Decalcification of Bone, etc.

1 gram of phloroglucin.
10 c.c of pure nitric acid (sp. gr. 1·4).

Mix and warm slowly and carefully. Dilute the resulting clear solution with 100 c.c. of distilled water and add 10 c.c. of pure nitric acid.

Tissues are decalcified very rapidly.

Excellent results may also be obtained by taking 1 gram of phloroglucin to 100 c.c. of from 5 to 40 per cent. pure hydrochloric acid.

Müller's Fluid.

Bichromate of potash,	2 parts.
Sulphate of soda,	1 part.
Water,	100 parts.

Mayer's Hæmalum.

Dissolve 1 gramme of hæmatin in glycerine by rubbing up in a mortar, and make up to 1000 c.c. with ·5 per cent. potash alum solution.

Kulschitsky's Hæmatoxylin.

Dissolve 1 gramme of hæmatoxylin in 10 c.c. of absolute

alcohol, and make up to 100 c.c. with 2 per cent. solution of acetic acid.

Pal's Differentiating Fluid.

Oxalic acid,	1 gm.
Sulphite of potash,	1 gm.
Distilled water,	100 c.c.

Jenner's Stain.

Grübler's water-soluble eosine, 1·2 per cent. solution in water,
Grübler's medicinal methylene-blue, 1 per cent. solution in water,
} equal parts.

These are thoroughly mixed in an open dish and left for 24 hours. Filter and dry in an oven at 50° C.

For use, ·5 gramme of the powder is thoroughly shaken up in 100 c.c. of pure methyl-alcohol and filtered. (The powder can be purchased.)

Hayem's Solution for Blood.

Corrosive sublimate,	·5 gms.
Sodium sulphate,	5·0 ,,
Sodium chloride,	1·0 ,,
Distilled water,	200·0 c.c.

Farrant's Medium.

Saturated solution of arsenious acid (filtered),
Glycerine,
Water,
} equal parts.

Add gum acacia until medium is of proper consistency, and then add 1 drop of carbolic acid.

Sugar Mounting Fluid.

Candied sugar,
Distilled water, equal parts,
} 4 parts.

Dextrin,
Distilled water, equal parts,
} 1 part.

Mix and add thymol.

Fixing Solution for Golgi's Method (Cox's Modification).

Potassium bichromate,	2 per cent.,	-	50 parts.
Potassium chromate,	5 ,,	- -	16 ,,
Corrosive sublimate,	5 ,,	- -	20 ,,

Reducing Solution for Cajal's Method.

Pyrogallic acid,	- -	1 gm.
Formol,	- - -	10 c.c.
Alcohol,	- - -	10 c.c. or so.
Distilled water,	- -	100 c.c.

GLASGOW: PRINTED AT THE UNIVERSITY PRESS BY ROBERT MACLEHOSE AND CO. LTD.